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Epidemiology

Risk factors for HPV infection and cervical cancer among unscreened women in a high-risk rural area of China

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ABSTRACT

We report a prevalence rate of 23.6% human papillomavirus (HPV) infection with oncogenic subtypes and 2.4% cervical intraepithelial neoplasia (CIN) III and cervical cancer (CC) in rural middle-aged women in 2 counties with the highest CC mortality in Shanxi Province, China. We examined the association of risk factors to HPV infection and to CIN III and CC in 8,798 unscreened women aged 35-50 years. Multivariate odds ratios (OR) and 95% confidence intervals (CI) for each endpoint were obtained for risk factors after adjustment for covariates. The OR of oncogenic HPV were: 1.41 (95% CI = 1.25-1.60) and 1.42 (95% CI = 1.24-1.61) for the participant and her husband having multiple sexual partners, respectively; 1.67 (95% CI = 1.37-2.04), 1.15 (95% CI = 1.04-1.26), and 0.82 (95% CI = 0.72-0.94) for ever (*vs*. never) diagnosed with tuberculosis, cervical inflammation and vaginal trichomoniasis, respectively; while bathing in a public (v. private) facility had an OR of 1.23 (95% CI =1.11-1.35). Seasonal fluctuations in HPV infection, but not CC, appeared in Xiangyuan County, with OR of 1.23 (95% CI = 1.14-1.33) and 1.51 (95% CI = 1.35-1.67) in Spring and Winter compared to Summer, respectively. The OR of CIN III and CC in the HPV positives were: 2.03 (95% CI = 1.63-2.53) for ages ≥45 years (*vs*. <40); and 4.01 (95% CI = 1.46-11.0) for ≥3 (*vs.* no) home births. Public health interventions and control strategies for improving the reproductive health of women in these rural populations need to be developed to reduce risk

of HPV and subsequent CC.

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ARTICLE TEXT

Wide disparities in cervical cancer (CC) incidence and mortality throughout the world are mainly attributable to differences in access to programs that detect and treat pre-invasive disease of the cervix.[1] Although the CC mortality rate in China has declined markedly from 14.6 to 4.3/100,000 in the past 20 years,[2] some medically underserved rural areas without access to CC screening continue to experience high mortality. A prime example is Yangcheng County with a mortality rate of 52/100,000 in Shanxi Province, which has the highest CC mortality rate in China.[2] Cervical cancer represents a major health burden in poor rural areas like Shanxi Province located in the middle of China.

Although infection with oncogenic subtypes of human papillomavirus (HPV) has been clearly established as a necessary cause of CC,[3][4] the low incidence of CC in contrast with the extremely high lifetime cumulative incidence of cervical infection with HPV suggests additional etiologic co-factors are involved in CC pathogenesis.[5][6] Research on risk factors for CC or HPV infection has been carried out in many countries,[7][8][9][10] but only 2 earlier studies have examined risk factors for CC in China.[11][12] State-of-the-art techniques for detecting HPV DNA and biopsy-based diagnoses have changed since earlier reports in China, with the potential for changes in previous prevalence estimates of HPV infection and the identification of new epidemiologic risk factors.

In 2001, a medical and public health team from Cancer Institute/Hospital of Chinese Academy of Medical Sciences, Beijing, China in collaboration with The Cleveland Clinic Foundation (Cleveland, OH) began a CC screening study of women aged 35-50 years in 2 counties (Yangcheng and Xiangyuan) with the highest CC mortality rates in Shanxi Province, China. The Shanxi Province Cervical Cancer Screening Study II (SPOCCS II) was designed to compare the sensitivity and specificity of as well as the acceptability of the HPV self-test (self-sampled) with that of the HPV direct test (clinician-sampled) and liquid-based cytology;[13][14] and to examine risk factors and other co-factors associated with HPV infection and with cervical intraepithelial neoplasia (CIN) III and CC. The objectives of this paper are to describe the risk factors associated with each endpoint, with the intent of identifying potential preventive strategies.

Material and methods

Subject recruitment, study design, and sample

All women aged 35-50 years residing in 15 communes in the 2 counties were invited to join the study, which began in May 2001 and ended in June 2002. Women were residents of 6 communes in Yangcheng County and 9 communes in Xiangyuan County, that were 150 km apart. The women traveled 0.5-30 km over mountainous or hilly terrain to attend the hospital out-patient clinic for screening in each county. The study had a cluster sampling design with the commune as the unit for the cluster, with approximately 72% (n = 9,183) consenting to participate. Eligible subjects included all non-pregnant women within the targeted age-range, who had an intact uterus, no history of pelvic irradiation, and had not been screened for cervical neoplasia within the past 5 years. The Human Subjects Review Boards of the Cleveland Clinic Foundation and the Cancer Institute/Hospital of the Chinese Academy of Medical Sciences in Beijing approved the study.

At each local commune clinic, eligible women were greeted by a nurse, who explained the study, obtained informed consent, and provided instruction in cervico-vaginal self-sampling using the Digene Cervical Sampler for HPV DNA testing (Hybrid Capture-II, Digene Corp.). Each woman then provided a cervico-vaginal sample based on the instructions from the nurse. Approximately 10 months on average (*i.e.*, from 4-12 months) after the HPV self-sample by each woman, the women were screened for HPV and CIN/CC by 1 of 3 attending gynecologic oncologists under the direct supervision of a senior gynecologic oncologist from CICAMS who was on study at the county hospital clinic.[15] The study design was a cross-sectional, hospital outpatient, clinic-based screening, that included an in-person clinic interview to collect information on risk factors potentially associated with HPV infection, precursor lesions and CC using a cluster-sampling frame.

Among 9,183 women who were enrolled in the initial phase (the HPV self-test) of the study, 8,519 (93%) attended the hospital clinic, and 664 women did not attend due to: refusals (4.6%), out-migration (0.4%), pregnancy (0.2%), death (0.1%) and other reasons (1.7%). At the same time as the 8,519 women attended the hospital clinic, another 515 women, who were initially missed, were enrolled from the same communes and brought to 1 of 2 clinics. Compared to the 8,519 women who participated in the self- and direct-tests, the 7% of women (n = 664) who completed the self-test, but did not get screened by gynecologists at the hospital outpatient clinic visit, and the 515 women who had a clinic screen but did not perform the self-test, had similar age, marital status, education and current smoking status. Specifically, the average ages for the 8,519 women with both tests, the 664 with the self-test only, and the 515 with the direct-test only were: 40.9 ± 4.4, 41 ± 4.8, and 41.4 ± 4.2 years, respectively. The percent married was: 98, 97, and 98, respectively, whereas the percentage of current smokers was: 3.4, 4.8, and 4.2; and 5-7 percent of each group had no formal education. The 515

women were compared to the 8,519 women on rates of HPV positivity, CIN II-III and CC, with the former group having rates of 20.8%, 3.3% and 0.4%, respectively, whereas the latter group had rates of 23.7%, 4.1% and 0.2%, respectively. Because no differences in the risk factors associated with each endpoint appeared in the models that included or excluded these 515 additional women, all analyses included these women. All women (n = 9,034) had the CC screening examination, but 8 women did not complete the interviewer-administered questionnaire; another 227 of the 8,519 women and 1 woman among the 515 women did not meet the age eligibility criterion. After exclusion of these 236 women, 8,798 remained with completed interviews and biospecimen assessments for data analysis.

Study Questionnaire and specimen collection

The risk factor questionnaire was pre-tested and administered in an earlier study of women of similar ages in Shanxi Province in 1999.[16] At the hospital clinic visit, each woman was asked about socio-demographic characteristics, sexual and reproductive history, history of infections, family history of cancer and lifestyle behaviors by trained health workers in a confidential setting before the clinical examination.

Cervical samples were collected for cytology and HPV DNA testing, and the criterion standard determined by random and colposcopically-directed biopsy. Details were described in an earlier study.[13][15] A diagnosis of atypical squamous cells of uncertain significance (ASCUS) or higher was considered positive for a pap test. The specimens were evaluated for presence of high risk oncogenic HPV subtypes using the Digene second generation hybrid capture assay.[17] The mixed probes can detect 13 oncogenic risk HPV types, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. A value of 1.0 pg/ml HPV DNA or more was used as the cut-off for positives for both the self-test and direct test. Each woman who was positive for HPV by self-test or direct test or for a pap smear underwent a colposcopy and biopsy. All cervical cytology, cervical biopsy and endocervical curettage specimens were read first by a junior pathologist, then by a senior pathologist, and the final diagnosis was the worst histologic diagnosis on any biopsy identified by the senior pathologist. Of 8,798 women, 3,631 had either a positive self-test or direct test for HPV, or a liquid-based cytology smear of ASCUS or worse; 3,098 had and 533 did not have the colposcopy and biopsy, respectively. Of 533 without specimens, 489 had ASCUS with a negative HPV test and were not recalled. Despite great efforts, 44 women with ASCUS and a positive HPV test did not return to the clinic for the clinical screening procedures.

Statistical analysis

The statistical analysis involved several steps. In the first step, estimates of the means and proportion of all known or suspected risk factors for HPV infection (based on the HPV direct test), CIN and CC were calculated. Prevalence rates of HPV oncogenic subtypes, and of CIN I, II and III and CC were calculated. The Pearson χ^2 test for independence was calculated to detect statistically significant differences in the proportion of women who had a risk factor among the HPV positives (*vs.* negatives) and among women with CIN III and CC in contrast with women who did not have CIN. The seasons were defined as: Spring (March-May), Summer (June-August), Fall (September-November), and Winter (December-February). The seasonal rates of screening in Xiangyuan County (n = 5661) were 76% in Spring, 10% in Summer, and 14% in Winter. No screening occurred in the fall. Screening in Yangcheng County (n = 3,137) occurred in May (53%) and June (48%). Menopausal status included: premenopausal (women who had a last menstrual period [LMP] $\leq$1 month of the clinic visit); postmenopausal (women reported LMP >1 year before the visit); and perimenopausal (women reported LMP >1 month but $\leq$1 year).

In the second step, the association between each risk factor and the odds ratio (OR) of an adverse endpoint was examined in univariate logistic regression analysis. All variables that were significantly associated with each endpoint based on the univariate logistic regression analyses were then entered in a multiple logistic regression model to adjust for all other variables. The OR of the following endpoints were examined: HPV positives for oncogenic subtypes; and the combined endpoint of CIN III and CC in all and in HPV-positive women. The percentage of women who were infected with HPV oncogenic subtypes or presented with CIN III and CC was compared to the HPV-negative women and those without CIN as reference groups, respectively. All analysis were computed using the software package PC-SAS callable SUDAAN release 8.0,[18] which accounted for the correlation between observations from the same commune due to the cluster sample design. The test for linear trend was calculated by scoring the categories of each variable in increasing order with integer values, and then treating the scores as a continuous variable in the logistic regression model. The p-values for trend are presented in the tables.

Potential confounding effects and effect modification were examined for: current age, smoking, and parity; ages at menarche and at first intercourse; lifetime number of sexual partners in the woman and in her spouse; marital status; menopausal status; induced abortions; education; place of delivery (home births *vs.* none); birth control method (tubal ligation *vs*. any other method); reported diagnosis of infections; season of screening; county of residence; history of cancer in the index patient and her family. Effect modification was analyzed by 2 methods: (*i*) restricting analyses to individual categories of selected covariates, *e.g.*, HPV-positive women; and (*ii*) including interactions between potential confounders and risk factors in the logistic regression models. All analyses adjusted for the other variables reported in the tables. All analyses were done using the software SAS 8.0.[19] Statistically significant findings were determined by a 2-tailed p-value of $\leq$0.05.

Results

Among 9,034 women who attended the hospital clinic, 8,798 women met the eligibility criteria and completed an interviewer-administered questionnaire. Most (96%) of the women were farmers. Five percent of the women had no formal schooling, 30% had primary school, 50% had junior high school and the remaining 15% had more than junior high school education. Their mean age at menarche was 15 years, with 21% reporting a 1-year interval from the age at first menses until regular menstrual cycles, and another 7% reporting >2 years to establish a regular menstrual cycle. They reported an average of 3 pregnancies and 2 live births, with 1% nulliparous, 9% primiparous and the majority (90%) multiparous. Among 93% of the women who delivered at home, 28% had at least 3 home births. Seventy-nine percent had a tubal ligation as their means of birth control, and 6% reported being post-menopausal, with an average age of menopause of 46 years. Another 7% and 87% of women were peri-or pre-menopausal, respectively.

Ninety-eight percent of the women were married, with their mean age at first sexual intercourse of 20 years. Twenty-five percent of the women reported having multiple sexual partners, whereas 15% reported their husbands having multiple sexual partners. A little over 3% were current cigarette smokers. The rates of reported ever diagnosis of tuberculosis (TB), cervical inflammation and vaginal trichomoniasis (v. trichomoniasis) were 3%, 34% and 21% respectively. The mean age at diagnosis of TB was 25 years with a range of 1-46 years. The median frequency of cervical inflammation or v. trichomoniasis was the same, notably once with an interquartile range of 1-2 times. Approximately 23.6% (2,077/8,798) of the women were positive for the oncogenic subtypes of HPV infection. Women who were positive and women who were negative for oncogenic HPV infection had the same median/mean age of 41 years (SD ± 4.3 or 4.3, respectively). HPV infection rates varied by season in Xiangyuan County, but not in Yangcheng County, with significantly lower rates in Summer (18.6%; 95% CI = 15.4-21.7) than in Spring (23.7%; 95% CI = 23.68-23.72) and Winter (26.1%; 95% CI = 23.1-29.1) (Fig. 1). Women who were screened in each season did not differ by demographic characteristics (data not shown). Based on biopsy results, 4% and 2% of the women were diagnosed with CIN I and CIN II, respectively. Another 2.1% of the women were diagnosed with CIN III and 0.3% with CC. Ninety-seven percent of CIN III and CC was positive for the oncogenic HPV infection. There was no seasonal variation in rates of CIN III and CC (data not shown). The median ages of HPV-positive women were: 40 years, with a mean of 41 ± 4.2 years in those without cervical disease; 41 years, with a mean of 41 ± 4.2 years in women with CIN I or II; and 43 years, with a mean of 42.5 ± 4.2 years in women with CIN III/CC.

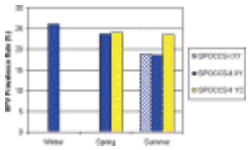

Figure 1. Variation in seasonal HPV prevalence rates in SPOCCS-I and SPOCCS-II. The oncogenic HPV prevalence rates in Xiangyuan County were 18.6%, 23.7%, and 26.1% in Summer, Spring and Winter, respectively (Pearson χ^2 = 7.24, *p* = 0.007). XY, Xiangyuan County; YC, Yang- cheng County.
[Normal View 30K | Magnified View 78K]

Multiple logistic regression models of HPV

In Table I, the odds of HPV were 41% or 42% higher for women having multiple sexual partners or their husbands having multiple sexual partners; 23% higher for bathing in a public facility; 67% higher in those ever having a diagnosis of TB, 15% higher for having a diagnosis of cervical inflammation, 18% lower for ever having a diagnosis of v. trichomoniasis. When the variable indicating ever/never diagnosed with cervical inflammation or v. trichomoniasis was replaced by the reported frequency of diagnoses of each infection, the OR of HPV remained statistically significant (OR = 1.05, 95% CI = 1.02-1.08 for cervical inflammation and OR = 0.93, 95%CI = 0.87-0.99 for v. trichomoniasis). In an analysis restricted to women from Xiangyuan County, the OR of HPV in women who were screened in Spring was 1.23 (95% CI = 1.14-1.33) and in Winter was 1.51 (95% CI = 1.35-1.67) compared to women screened in Summer. In an analysis that added county of residence to the overall model of HPV positives for oncogenic subtypes, the OR do not appreciably change (*i.e.*, only in the hundredth decimal place) for most variables, except for the OR for winter that became significant and increased to 1.33 (1.09-1.61), whereas the OR for county of residence did not reach statistical significance.

Table I. Adjusted or of HPV Infection in Spoccs-II

	Oncogenic HPV Positive *n* = 2,077 (%)	Oncogenic HPV Negative *n* = 6,721 (%)	OR[a] (95% CI)
Multiple sexual partners of the husband			
No	1,666 (80.2)	5,835 (86.8)	1.00
Yes	411 (19.8)	886 (13.2)	1.42 (1.24-1.61)
Multiple sexual partners of the woman			
No	1,439 (69.3)	5,203 (77.4)	1.00

Yes	638 (30.7)	1,518 (22.6)	1.41 (1.25-1.60)
Current smoking			
No	1,991 (95.9)	6,504 (96.8)	1.00
Yes	86 (4.1)	217 (3.2)	1.17 (0.88-1.56)
Bathing location			
Home	1,128 (54.3)	3,875 (57.7)	1.00
Public house	949 (45.7)	2,846 (42.3)	1.23 (1.11-1.35)
Post-menopausal			
No	1,784 (92.6)	5,856 (93.9)	1.00
Yes	143 (7.4)	384 (6.1)	1.22 (0.99-1.49)
Stillbirths			
0	1,948 (93.8)	6,195 (92.2)	1.00
1	104 (5.0)	434 (6.4)	0.75 (0.60-0.94)
2+	25 (1.2)	92 (1.4)	0.88 (0.50-1.55)
p-value for trend			0.03
Tuberculosis			
No	1,995 (96.1)	6,564 (97.7)	1.00
Yes	82 (3.9)	157 (2.3)	1.67 (1.37-2.04)
Cervical inflammation			
No	1,321 (66.7)	4,485 (63.6)	1.00
Yes	756 (33.3)	2,236 (36.4)	1.15 (1.04-1.26)
V. trichomoniasis			
No	1,675 (80.6)	5,278 (78.5)	1.00
Yes	402 (19.4)	1,443 (21.5)	0.82 (0.72-0.94)
Season			
Summer	461 (22.2)	1,616 (24.0)	1.00
Spring	1,407 (67.7)	4,514 (67.2)	1.00 (0.84-1.18)
Winter	209 (10.1)	591 (8.8)	1.15 (0.96-1.39)
p-value for trend			0.25

[a] Each OR was adjusted for all other variables presented in the table using the program, SUDAAN in a multiple logistic regression analysis.

Multiple logistic regression models of CIN III and CC

In Table II, the odds of CIN III and CC increased with increasing age and number of home deliveries, but decreased with increasing educational level. The odds of CIN III and CC were 44% and 29% higher for ever being diagnosed with TB and cervical inflammation, respectively, and 38% lower for ever being diagnosed with v. trichomoniasis, but these OR probably did not reach statistical significance because of the lower rates of CIN III and CC than HPV. Nonetheless, the magnitudes of the OR for CIN III and CC by infection were similar to the magnitudes for HPV by infection. Note that the risk estimate (OR = 166; 68-405) for oncogenic HPV infection of CIN III and CC was observed (Table II, Model: OR[a]). In an analysis that added county of residence to the model of CIN III/CC, the effect of home births is reduced: to 1.60 (0.79-3.24) in those with one home birth; to 1.79 (0.80-4.04) in those with 2 home births; and to 3.16 (1.19-8.36) in those with 3 or more home births. In the model restricted to HPV-positive women, subject's age and home births were still positively associated with CIN III and CC, with a significant increasing trend for each (Table II, Model: OR[b]). Interactions between bathing location and genital washing after coitus, the participant's and her husband's multiple sexual partners, cervical inflammation and v. trichomoniasis, were tested but were not statistically significant in the models. Potential confounding variables that were not statistically significantly associated with any endpoint included: age at menarche and at first intercourse; alcohol consumption; history of cancer in the index woman and in her family; gravidity; marital status; history of induced abortions and educational level of the woman.

Table II. Adjusted or of CIN III and Invasive Cervical Caner, Spoccs-II

	CIN III and CC *n* = 212 (%)	Normal[c] *n* = 8,062 (%)	OR[a] (95% CI)	OR[b] (95% CI)
Age				
35-39	68 (32.1)	3,560 (44.2)	1.00	1.00
40-44	64 (30.2)	2,517 (31.2)	1.20 (0.88-1.63)	1.21 (0.89-1.65)
45-50	80 (37.7)	1,985 (24.6)	1.96 (1.58-2.43)	2.03 (1.63-2.53)
p-value for trend			<0.001	<0.001
Education				
≤Primary school	90 (42.5)	2,787 (34.6)	1.00	1.00
Junior high school	99 (46.7)	4,090 (50.7)	0.87 (0.65-1.18)	0.87 (0.64-1.19)
>Junior high school	23 (10.8)	1,185 (14.7)	0.72 (0.44-1.15)	0.75 (0.46-1.22)
p-value for trend			0.01	0.002
Parity				
0	2 (0.9)	102 (1.3)	1.00	1.00
1	14 (6.6)	851 (10.6)	0.41 (0.06-2.60)	0.46 (0.07-3.21)
2	128 (60.4)	4,697 (58.3)	0.52 (0.09-3.01)	0.50 (0.09-2.86)
3+	68 (32.1)	2,412 (29.9)	0.25 (0.04-1.53)	0.24 (0.04-1.45)
p-value for trend			0.14	0.32
Home births				
0	7 (3.3)	568 (7.1)	1.00	1.00
1	23 (10.8)	1,207 (15.0)	1.81 (0.84-3.89)	1.59 (0.78-3.25)
2	108 (50.9)	4,018 (49.8)	2.13 (0.95-4.79)	2.16 (0.90-5.19)
3+	74 (34.9)	2,269 (28.1)	3.91(1.48-10.3)	4.01 (1.46-11.0)
p-value for trend			0.002	0.001
Multiple sexual partners of the husband				
No	172 (81.3)	6,932 (86.0)	1.00	1.00
Yes	40 (18.9)	1,130 (14.0)	0.91 (0.57-	0.89 (0.55-

			1.46)	1.45)
Multiple sexual partners of the subject				
No	145 (68.4)	6,138 (76.1)	1.00	1.00
Yes	67 (31.6)	1,924 (23.9)	1.06 (0.68-1.62)	1.11 (0.73-1.69)
Tuberculosis				
No	201 (94.8)	7,853 (97.4)	1.00	1.00
Yes	11 (5.2)	209 (2.6)	1.44 (0.65-3.22)	1.47 (0.64-3.37)
Cervical Inflammation				
No	129 (60.8)	5,346 (66.3)	1.00	1.00
Yes	83 (39.2)	2,716 (33.7)	1.29 (0.96-1.74)	1.21 (0.88-1.66)
V. trichomoniasis				
No	181 (85.4)	6,338 (78.6)	1.00	1.00
Yes	31 (14.6)	1,724 (21.4)	0.62 (0.38-1.01)	0.60 (0.35-1.02)
Oncogenic HPV(+)				
No	6 (2.8)	6,657 (82.6)	1.00	
Yes	206 (97.2)	1,405 (17.4)	166 (68-405)	

[a] Each OR was adjusted for all other variables presented in the table using the program, SUDAAN in a multiple-logistic regression -analysis.

[b] Restricted to the HR-HPV positive women, and adjusted for all variables in table using SUDAAN multiple logistic regression.

[c] No CIN detected in cervix.

Discussion

As part of a screening study of women aged 35-50 years in 2 counties with the highest CC mortality in Shanxi Province, China, we report the prevalence rates of 23.6% HPV infection for oncogenic subtypes; 2.4% of these women were diagnosed with CIN III and CC. Seasonal variation in HPV prevalence, but not CIN III and CC, rates appeared in one county where screening occurred in 3 different seasons, but not in the other county where screening occurred in 2 consecutive months. Sexual habits of both spouses and unhygienic practices, as well as history of TB and cervical inflammation raised the risk of HPV infection and CIN III and CC. In contrast, diagnosis of v. trichomoniasis reduced the odds of HPV infection and CIN III and CC. This finding might be an indicator of treatment for v. trichomoniasis or access to health care. Once the model of CIN III and CC was restricted to the HPV positives, sociodemographic characteristics remained significant factors.

The prevalence rate of oncogenic HPV infection of 23.6% was much higher than population-based prevalence rates of: 11.4% in Colombian[20] women ranging in ages from <20 years to ≥55 years; 7.6% in Costa Rican[21] women aged 18-94 years; and 4-12% in Mexican women aged 35-64 years.[22] In Asian surveys, the prevalence rates of all HPV infections were: 10% in South Korean women aged 20-74 years;[23] 10.9% in South Vietnamese women ranging in ages from <25 to ≥65 years;[24] and 6.3% in women aged ≥15 years in Thailand.[25] Therefore, earlier population-based rates of HPV infections included women of a wider range of ages associated with varied HPV rates and access to CC screening.

The rates of HPV positivities and of CIN II+/CC in this rural Chinese population were similar to the rates of 21.3% and 4.0%, respectively, in women aged 35-65 years in rural South Africa.[26] The rates of HIV positives differed in the 2 populations, with 8% HIV positive in 2,944 South African women[27] in contrast with no HIV positives in the earlier SPOCCS I study.[28] It seems that the high rates of HPV, CIN and CC in the South African series were, in part, due to the HIV-positive rate, because persistence of HPV is much more likely in HIV-positive than in HIV-negative women.[29]

The overall rate of HPV positives in SPOCCS II was significantly higher than the 18% prevalence rate in SPOCCS I conducted in other parts of one (Xiangyuan) of 2 counties ($\chi^2 = 6.8$, $p = 0.01$), whereas the rates of CIN II+ were similar (4.4% *vs*. 4.3%).[30] The rates of HPV infection in SPOCCS I and II were 18% and 18.6%, respectively among women in Xiangyuan County screened in Summer, however, thereby removing the season and county effects (Fig. 1). The HPV prevalence rates were not statistically different between Spring and Summer in the other (Yangcheng) County, because the samples were collected during 2 consecutive months at the end of Spring and early Summer in this county rather than

across all 6 months of Spring and Summer as in Xiangyuan county. Both studies reported oncogenic subtypes for HPV infection using the Hybrid Capture II, recruited women who had not been screened for CC in the past 5 years, and employed similar design and specimen collection procedures. The seasonal variations in HPV prevalence rates in Xiangyuan County showed lower rates in Summer than in Spring and in Winter. One earlier study in the Netherlands reported seasonal variation in the prevalence rate of HPV infection over a 9-year period, with the highest rates in Summer, lower rates in Fall and in Spring and the lowest in Winter; rates of HPV and CC were significantly higher in Summer than Winter.[31] A Mexican study reported higher rates of CC from the end of Fall to the beginning of Winter.[32] In contrast, we report no seasonal variation in detection of CC. Yet all cross-sectional research, including the current study, of the seasonal variation of HPV and CC suffers from an inability to detect seasonal variation in the onset of infection.

Recognition of the necessary, but not sufficient, causal role of HPV in CC has encouraged epidemiologists to focus on co-factors contributing to carcinoma. In addition to seasonal variation in HPV, the risk factors that increased the odds of HPV infection included sexual behavior and history of TB, and cervical inflammation. V. trichomoniasis lowered the odds of HPV. Similar to other studies,[33][34] our results demonstrated that multiple sexual partners of the participant or spouse played an important role in HPV transmission. Sexual behavior was not associated significantly with CIN III and CC, a finding that was similar to one study[35] but not another after adjustment for HPV infection.[36] Because the participant reported her husband's sexual behavior, the role of and contribution of male promiscuity in HPV infection was perhaps underestimated in our study.

Certain hygiene habits, notably the location where the women bathed, were associated with HPV infection. Specifically, washing facilities at home presumably protected women from HPV infection in contrast with taking a shower in the public bathhouse. This might be related to the incommodious space and poor hygienic conditions of the public bathhouse in rural areas or bathing may have occurred less frequently in those required to travel to the public bathhouse in winter weather conditions. There is some epidemiological evidence to suggest that genital tract disease such as cervical inflammation might be linked to CC or high-grade lesions.[37][38][39] This association was observed for HPV infection in our study.

Smoking, a well-known risk factor for cervical cancer, was reported in 3.4% of the women and was associated with a 17% (significant) increased risk for HPV in the univariate, but not in the multivariate analysis for HPV. The magnitude of the effect for smoking was smaller than observed in earlier research,[5] perhaps due to the relatively small proportion of women who smoke in this culture. This is an indication that smoking may be a relatively uncommon habit of limited duration in rural China or an economically prohibitive practice.

Two infections were consistently associated with HPV infection, notably a history of TB was associated with a higher odds of HPV infection, whereas history of V. trichomonosis was inversely associated with HPV infection. There was no correlation between TB and socioeconomic status in our data, which suggests host immunity might play an important role in the occurrence of HPV to be discussed in a future manuscript. At first glance, it might seem surprising that v. trichomoniasis emerged as a protective factor. Indeed v. trichomonas may act as co-factor of HPV exposure in carcinogenesis.[10][12][40] For example, in a meta-analysis of v. trichomoniasis and CIN I-III plus CC, the OR was 1.93 (1.22-2.65) in 2 cohort studies, whereas the OR in 22 case-control studies was much less consistent.[42] In our study, we did not collect data on treatment for infection. One of the co-authors (F.H.Z.) contacted health care providers at the local hospital, who stated they prescribe the medicine (Metronidazole) to treat women for v. trichomoniasis. It is also important to note that women reported culturally-recognized symptoms for v. trichomoniasis, which may be an indicator of access to health care. Metronidazole, a synthetic antimicrobial drug, might inhibit the growth of other genital co-factors by modulating the vaginal environment from an anaerobic to an aerobic one in women with HPV or reduce nitrosamine or prostaglandin biosynthesis.[43] To date no reported *in vitro* or *in vivo* data have investigated the potential effect of metronidazole on HPV infection. Metronidazole needs to be further evaluated because it is a proven mutagen in bacterial systems, is genotoxic to human cell lines, and is carcinogenic in animal model systems.[44] Because the drug is being used more and more frequently across the world, there is a need for improved studies to elucidate potential mechanisms of genotoxicity and its carcinogenic potential. Further studies of TB and sexually transmitted diseases are needed to explore underlying mechanism of the risk of HPV infection.

The magnitude of the OR for CIN III and CC varied for infections when HPV positives were in or out of the models. Older age of women, and delivering newborns at home were consistent risk factors associated with CIN III and CC in all models with a significant positive trend. The increased risk of CIN III and cancer with increasing age was found in the Kaiser-Fontana population in a review from 1996.[45] Giving birth at home is an indicator of the lack of or limited use of health care services for multiple conditions. Thus, once woman aged 35-50 years had HPV oncogenic subtypes, then indicators of disparities in access to health care were the factors associated with risk of CIN III and CC. Indeed contraception or family planning programs are accessible annually to women in China, but the same women do not have access to HPV and CC screening. Perhaps disparities in CC screening could be remediated by incorporating Pap smear with family planning.

Our current study has several limitations and strengths. The cross-sectional design limits the ability to interpret whether co-factors are causal and the timing of their contribution to the carcinogenesis progression. Reliance on reported infections might have led to an underestimate of infectious disease, which could also be confounded by access to health care and SES. Cervical inflammation is a poorly defined condition, usually including polyps that spontaneously regress. The analysis

identified an array of oncogenic subtypes for HPV infection but did not distinguish individual subtypes. Finally, the sample may not be representative of the total population of women aged 35-50 years in each county, because 72% of women who were invited actually participated in the self-sample biospecimen collection. Another 515 were missed originally but participated along with 93% of those who carried out the self-sampling procedure. When we compare the 515 women originally missed with those who participated in both the self- and clinician-based screening, there are no differences in the mean age nor in the percent with: HPV infections; CIN I, II, or III/CC; home births; tubal ligations; or by educational level. Thus, the 515 seem to be comparable to the larger group of participants on SES, indicators of access to care, disease endpoints, and age. Nonetheless, we cannot exclude the possibility that some bias might have arisen from the 28% of women who did not participate in the self-sampling or the clinic-based screening. As regards study strengths, all women had not been screened within the past 5 years at a minimum. Biopsies and colposcopies were carried out on women who had been identified as HPV-positive or ASCUS by pap smear and returned for follow-up visit. Only a small proportion of women did not return for the follow-up visit. The interview was conducted in a private room using a previously piloted questionnaire in a similar population.

In summary, our study was conducted in middle-aged women with limited access to screening in low resource counties recognized for high CC mortality in rural, mid-west China. An array of factors related to sexual practices, hygienic conditions, and multiple infections were associated with the odds of HPV infection. Once the analysis was restricted to women positive for HPV infection, however, demographic characteristics, such as age (an indicator of length of infection) and SES indicators remained in the model of CIN III and CC. Our study suggests a role of season in the presence of HPV infection, and further studies are needed to address seasonality of onset and of factors influencing the relationship between season and HPV infection. Our study findings may have implications for the planning of specific preventive strategies aimed at reduction of CC risk and merged with family planning program. Public health intervention and control strategies for improving women's genital reproductive health need to be integrated as a high priority in this rural population.

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